Review of Stem Cell Studies

Ma Hongbao *, Cherng Shen **

* Brookdale University Hospital Medical Center, Brooklyn, New York 11212, USA,
hongbao@gmail.com; 347-789-4323

** Department of Electrical Engineering, Chengshiu University, Niaosong, Taiwan 833, Republic of China,
cherngs@csu.edu.tw; 011886-7731-0606 ext 3423

Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life and all cells come from stem cells. Germline stem cell (GSC) is the cell in the earliest of the cell stage. This article is a review of the stem cell research to introduce the current topics in the stem cell field. [Nature and Science. 2007;5(2):45-65] (ISSN: 1545-0740).

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1. Introduction

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" *(*Stedman's Medical Dictionary, 2002).

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into all different types of cells in life bodies, tissues and organs. Stem cells can be used to the study of the essential properties of the life, and it can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003), and also it gives a hope to let us get the eternal life. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

The long-term implications of stem cell therapy could be a revolution in medicine. Many diseases are caused by the death of cells vital to the proper functioning of the organs. Heart failure, for example, is often caused by damage to the muscles caused by a blood clot. Stem cells injected into the heart could recreate the heart muscle. Type 1 diabetes is caused by the destruction of the pancreatic cells that make insulin. These cells might be reintroduced as stem cells. Parkinson's disease is caused by a loss of cells. In animal experiments stem cells have been shown to reduce symptoms of the disease.

Some of the most notable recent findings are as follows:

(1) The stemness profile may be determined by approximately 250 genes;

(2) organ-specific stem-cell growth and differentiation are stimulated during the reparative phase following transient injury;

(3) Two bone marrow stem-cell types show a remarkable degree of differentiation potential;

(4) Some organs contain resident marrow-derived stem cells, and their differentiation potential may only be expressed during repair;

(5) The metanephric mesenchyme contains pluripotent and self-renewing stem cells;

(6) Marrow-derived cells invade the kidney and differentiate into mesangial and tubular epithelial cells, and these processes are increased following renal injury;

(7) Epithelial-to-mesenchymal transition generates renal fibroblasts (Oliver, 2004).

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing

totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

The animal body has an unlimited source of stem cells, almost. However, the problem is not in locating these stem cells, but in isolating them from their tissue source.

Five key stem cells have been isolated from human:
(1) Blastocysts;
(2) Early embryos;
(3) Fetal tissue;
(4) Mature tissue;
(5) Mature cells that can be grown into stem cells.

At least, three aspects attract people to be interested in stem cell:
(1) To explore the life mysteries;
(2) To cure disease;
(3) To extend human's life.

2. Germline Stem Cell (GSC)

The recently developed testis cell transplantation method provides a powerful approach to studying the biology of the male germline stem cell and its microenvironment, the stem cell niche. For example, the *Drosophila* testis contains an average of nine germline stem cells surrounding a small cluster of nondividing somatic cells known as the hub. Two recent studies have shown that the hub is responsible for creating the germline stem cell niche by secreting a signal that is required by germline stem cells for their self-renewal. Testis is the organ for animal to reproduce the generation. As the new generation always has a young feature for the life, no matter how old the parents are, it is possible for the mature life to use the stem cell coming out from the reproduce organ (germline stem cells) to replace the old cells, to keep the mature body always young. In *Drosophila* germline stem cells of the testis, one centrosome remains anchored to the region of the cortex at the interface between germ cells and somatic hub cells, while the other centrosome migrates to the opposite side to establish mitotic spindle orientation. The orientation of the mitotic spindle ensures that as the stem cell divides, the daughter cell nearest the hub remains in the niche and is marked for self-renewal, whereas the daughter cell farther away from the hub is edged out of the niche and begins to differentiate.

Germline stem cells in *Drosophila* testes that carry a mutation in centrosomin, an integral centrosome component, provide clues as to how the spindle-positioning mechanism may operate. These mutant stem cells display defects in positioning of the centrosomes during interphase, and the resultant mitotic spindles are often misoriented. This is consistent with a direct role for the centrosomes in setting up the division plane, as suggested by the early localization of the centrosomes during interphase. Strikingly, the number of stem cells in the testes of the centrosomin mutant flies increases significantly. These stem cells become crowded around the hub, presumably because of the symmetric divisions of stem cells that have misoriented spindles. It thus appears that in *Drosophila* testes, the balance between stem cell self-renewal and differentiation is not dictated entirely by the amount of available space in the niche; rather, this balance is influenced directly by the orientation of stem cell division.

The study of stem cells holds immense promise for furthering our understanding of processes such as embryonic development, adult aging, and tumor formation. This is due to their remarkable ability to self-renew, to produce more stem cells and to differentiate into one or more specialized cell types. The *Drosophila* testis contains an average of nine germline stem cells surrounding a small cluster of nondividing somatic cells known as the hub.

The stem cells that sustain metazoan tissues face a difficult challenge. Each time a stem cell divides--it can divide indefinitely - it risks damage from errors in the duplication and segregation of genetic and cellular material that could stunt its vitality or propel it toward a cancerous state. Normally, each division must be asymmetric to ensure that only one daughter cell differentiates, while the other becomes a stem cell, thus renewing the stem cell population. Yet stem cells safely grow and divide many more times than other cell types, including their own daughters.

Unlike other known animal cell organelles, the two centrosomes inherited by daughter cells at division are not identical. All normal cells initially have one centrosome, comprising a mother and daughter centriole as well as pericentriolar material. The mother centriole contains structures and proteins that are

absent from the daughter centriole, and it nucleates more microtubules than the daughter. During each cell division cycle, the centrosome replicates. The mother centrosome retains the original mother centriole. In contrast, the daughter centrosome undergoes maturation during mitosis and during the G_1 phase of the next cell division cycle, converting its inherited daughter centriole into a new mother centriole. Whether this intrinsic asymmetry facilitates asymmetric stem cell division has remained a mystery.

Possible additional roles for programmed centrosome inheritance in stem cells. Aside from their participation in spindle assembly, centrosomes associate with membrane-bound organelles such as the Golgi and recycling endosomes. Centrosomes also regulate cytokinesis by delivering membranes asymmetrically to the cleavage furrow. Is differential centrosome inheritance the long-sought secret of stem cell, function? It should now be possible to determine whether maternal centrosomes are retained by several other well-characterized *Drosophila* stem cells. In male germline stem cells, such behavior seems likely to contribute to the stable asymmetric programming of stem cell and daughter. And it is satisfying to contemplate the possibility that this strategy might also promote stem cells' remarkable stability and longevity.

Extrinsic signals from niches are believed to control stem cell behavior, including self-renewal through interacting with intrinsic factors. However, it remains largely unclear how niche signals regulate their target gene expression in stem cells at the chromatin level. Adenosine triphosphate (ATP)–dependent chromatin remodeling factors control stem cell self-renewal by regulating responses to niche signals. Chromatin remodeling factors are involved in maintaining chromatin structures and modulating gene expression in organisms ranging from yeast to humans.

Male germline stem cells, called spermatogonial stem cells (SSCs) in postnatal mammals, are the foundation of spermatogenesis (the process for spermatozoa production) and, together with oocytes from females, are essential for species continuity. SSCs reside on the basement membrane of the seminiferous tubule in the testis and are almost completely surrounded by somatic Sertoli cells, which form a microenvironment or niche. Within the niche, growth factors and extracellular signals regulate the fate decisions of SSCs either to self-renew or to form daughter cells that will begin the complex differentiation process of spermatogenesis, resulting in mature spermatozoa after about 35 days in the mouse and 64 days in the human. The timing of sequential steps in spermatogenesis is tightly regulated by genes of the germ cell, and Sertoli cells support the differentiation process.

The first step in spermatogenesis is the fate decision of an SSC to produce daughter cells committed to differentiation. There is no known unique biochemical or phenotypic markers for distinguishing SSCs from their initial daughters, called undifferentiated spermatogonia. The availability of a functional transplantation assay and a culture system that allows long-term replication of SSCs made it possible to examine intracellular signals that influence self-renewal and differentiation invitro in a rigorous manner that is not available for most adult stem cells. These studies demonstrated that *Oct 3/4* and *SRY–box-containing gene 2* (*Sox 2*), which regulate *Nanog,* are expressed in SSCs. Stem cell recovery and cryopreservation may be applicable to all mammalian species and could be used to preserve the male germ line of valuable livestock animals, companion animals, and endangered species. Perhaps the most provocative and potentially valuable medical application of SSC research is for prepubertal boys undergoing chemotherapy or irradiation for cancer.

There are many possible future directions to pursue. Three particularly important areas include (1) the further definition of factors and signals that support self-renewal of SSCs, relative to those that initiate differentiation in order to provide a better understanding of this fate decision; (2) the extension of the serum-free culture system to other species, including domestic animals, endangered species, and humans to confirm that self-renewal signals are conserved among mammals and for relevant applications; and (3) the development of methods to allow in vitro differentiation of stem cells to provide mature spermatozoa, which would be enormously valuable in understanding the complex process of spermatogenesis and would have great practical use.

Stem cells are unique cell populations that are able to undergo both self-renewal and differentiation and are found in the embryo, as well as in the adult animal. In the early mammalian embryo, pluripotent embryonic stem cells are derived from the blastocyst stage and have the ability to form any fully differentiated cell of the body. As the embryo develops, stem cells become restricted in their ability to form different lineages (multipotent stem cells). Multipotent stem cells are also found in a wide variety of adult tissues such as bone marrow and brain. However, in the adult animal, the ability of certain stem cells to differentiate can be restricted to only one cell lineage (unipotent stem cells). Examples of mammalian

unipotent stem cells include the stem cells residing in the gut epithelium, the skin, and the seminiferous epithelium of the testis.

Spermatogonial stem cells can generate spermatogenesis when transplanted into the seminiferous tubules of an infertile male. Spermatogonial stem cells exhibit a distinct phenotype such as the high expression of ß-1 and specific light-scattering properties. While the stem cell identity of the A_s spermatogonia has not yet been rigorously demonstrated, their morphology and location in the seminiferous epithelium make them good candidates for being stem cells.

The ability to isolate, culture, and manipulate the germ line stem cell in vitro would allow us to unravel the molecular mechanisms that drive the first steps of spermatogenesis and to characterize the signaling pathways that induce spermatogonial differentiation versus self-renewal. In turn, this could help us understand the origin of certain testicular neoplasias and the causes of male infertility. To look at these issues, an in vitro system in which these cells could be maintained in long-term cultures would be ideal. In the study reported here, we attempted to establish a mouse spermatogonial stem cell line using the large T antigen under the control of an inducible promoter.

In the mammalian testis, the germ line stem cells are a small subpopulation of type A spermatogonia that proliferate and ultimately differentiate into sperm under the control of both endocrine and paracrine factors.

3. Embryonic Stem Cell

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

In 2003, scientists in Edinburgh have identified the gene that gives foetal stem cells their ability to multiply without limit and never grow old (Hawkes, 2003). The discovery may make it possible to create foetal stem cells from adult cells, and use them to treat diseases. At present the only way to get such cells is to create embryos. This is controversial, especially in the United States where federal research money cannot be used for embryonic research of this kind. The gene, which the team has named Nanog after the mythical Celtic land where nobody grows old, is a regulator that controls the operation of many other genes. It operates only in embryonic stem cells, which are pluripotent (able to develop into any of the body's specialised cells). Nanog's role, according to papers published in the journal *Cell* by the team from Edinburgh University and Nara Institute of Science and Technology in Japan, is to maintain stem cells and to make them grow. Ian Chambers, of the Institute for Stem Cell Research at Edinburgh, said that nanog was a master gene, which "makes stem cells immortal". Unlike specialised cells, that can only divide a limited number of times before they die, embryonic stem cells can go on dividing for ever. This means that a culture of stem cells can be kept alive for transplantation into patients where they will diversify into necessary cells — brain, muscle, liver or skin, for example. For this to be possible, scientists need to understand how it is that stem cells can either divide without limit, or choose instead to differentiate into specialised cells. Nanog appears to be the key. Nanog does not disappear in adult cells, but it lies dormant. This means that if a way could be found to reactivate it, adult cells could be persuaded to become embryonic cells again.

James Thompson, of the University of Wisconsin, told the *Washington Post*: "As we know`more and more about pluripotency, it will probably be possible to reprogramme cells to make stem cells out of any cell in the body. This is an important step in that direction." The Edinburgh paper is published alongside a study from Shinya Yamanaka, from the Nara Institute. The two groups realised that they had discovered the same gene last year and have since collaborated in completing the research. The next step is to work out how Nanog is switched on and off. To achieve that it may be necessary to continue working on embryonic stem cells and watching the process as it happens. British scientists have long argued that while work on adult stem cells is important, understanding how they work still requires the use of embryos. Most of the research so far has been conducted in mice, but humans have an almost identical gene. In one experiment the Edinburgh team inserted the human Nanog gene into embryonic mouse cells, and subjected those cells to conditions that would normally make them turn into specialist cells. The human Nanog gene stopped that process. Embryonic stem (ES) cells can be cultured in conditions that either maintain pluripotency or allow differentiation to the three embryonic germ layers. Heparan sulfate (HS).

4. Somatic Stem Cell

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

5. Isolation and Characterization of Stem Cells

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterization of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

5.1 Prostatic tissue collection and culture

When using human tissue, formal consent by the donator must be obtained before tissue collection. Tissue sections are obtained under sterile conditions. Each individual tissue section is bisected with half being sent for histological analysis for diagnostic evaluation and the remainder used for tissue culture. After then, tissue sections are chopped and placed in collagenase type I at 200 U/ml in RPMI 1640 medium with 2% v/v FCS overnight on a shaking platform at 37°C. The digest is then broken down further by shaking in 0.1% trypsin in PBS with 1% BSA and 1 mM ethylenediaminetetraacetic acid (EDTA) for 15-20 min. The cell suspension is then washed three times in PBS with 1% BSA and 1 mM EDTA before resuspending in RPMI 10% v/v FCS. Prostate epithelial cells are separated from fibroblasts by differential centrifugation (360 g, 1 min without braking). This process produced a supernatant enriched for fibroblasts and a pellet enriched for epithelia. The epithelial cell suspension is then spun on a metrizamide gradient (1.079 g/ml), and the cells are isolated from the interface (Bhatt, 2003).

5.2 Ber-EP4/α_2/CD45 labeling of cells

Isolated epithelial cells are labeled at ambient temperature with either anti-human integrin α_2 monoclonal antibody or Ber-EP4 antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Samples are then dual labeled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

5.3 Ber-EP4/α_2 and Hoechst labeling for flow cytometry

Isolated epithelial cells are labeled at ambient temperature with anti-human integrin α_2 monoclonal antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Hoechst staining could be performed by using the protocol for HSC as described by Rupesh, et al (Bhatt, 2003). Briefly, epithelial cells are resuspended in Hoechst buffer (Hanks' balanced salts solution, 10% FCS,`1% D-glucose, and 20 mM HEPES) and warmed to 37°C. Hoechst 33342 is then added to give a final concentration of 2 μM and the cells incubated at 37°C for 2 h. Fifteen min before the end of incubation, the cells are labeled with monoclonal anti-human Ber-EP4 directly conjugated to FITC (8 μg/ml). The cells are then washed in ice-cold Hoechst buffer before resuspending in ice-cold Hoechst buffer containing propidium iodide (PI) at 20 ng/ml (Bhatt, 2003).

5.4 Flow cytometry isolation of the SP fraction

Flow cytometry is carried out using a Becton Dickinson FACS Vantage SE flow cytometer. Hoechst 33342 is excited with an argon ion, ultraviolet-enhanced laser at 350 nm, and its fluorescence is measured with a 424/44 BP filter (Hoechst BLUE) and a 675DF20 BP optical filter (Hoechst RED; Omega Optical, Brattleboro VT). A 640 LP dichroic mirror is used to separate the emission wavelengths. PI fluorescence is also measured through the 675DF20 BP (having been excited at 350 nm). A second argon ion laser is used to excite the additional fluorochrome PE-Cy7 at 488 nM. PE-Cy7 is measured using a 787RDF40 (Omega Optical) filter (Bhatt, 2003).

5.5 Cell cycle characterization of SP fraction

Epithelial cells are isolated and all fractions are resuspended in Hoechst buffer and warmed to 37°C. Hoechst 33342 is then added to give a concentration of 2 μM and incubated at 37°C for 45 min. Pyronin Y (250 ng/μl) is added to each tube, and the samples are incubated for 45 min. Monoclonal anti-human Ber-EP4 FITC (8 μg/ml) is added as appropriate 15 min before the end. After this, ice-cold Hoechst buffer is added immediately and the samples are washed then resuspended in ice-cold Hoechst buffer. The samples are analyzed immediately by flow cytometry. Flow cytometry is performed using a modification of the method described above. Cells under study are selected by positive labeling for Ber-EP4 FITC before being analyzed for Hoechst and Pyronin Y staining. These cells are then analyzed by plotting the Hoechst profile on the x-axis and Pyronin Y along the y-axis in a linear scale (Bhatt, 2003).

5.6 Cytokeratin phenotype studies

Samples are processed as above, divided into two fractions, and labeled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labeled with Ber-EP4 FITC and integrin α_2 PE-CY7. Flow cytometry is performed as described and analyzed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

6. Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

Indeed, in many research projects, scientists have demonstrated that stem cells can be used to replenish or rejuvenate damaged cells within the immune system of the human body and that damaged stem cells can repair themselves and their neighbors. For example, in what is regarded as the first documented case of successful gene-therapy "surgery", scientists at the Necker Hospital for Sick Children in Paris of French succeeded in treating two infants diagnosed with Severe Combined Immunodeficiency Disease, a life-threatening degenerative disease caused by defects on the male (X) chromosome. With the identification of stem cell plasticity several years ago, multiple reports raised hopes that tissue repair by stem cell transplantation could be within reach in the near future (Kashofer, 2005). In cardiovascular medicine, the possibility to cure heart failure with newly generated cardiomyocytes has created the interest of many researchers (Condorelli, 2005). Gene clone techniques can be widely used in the stem cell researches and applications (Ma, 2004).

7. Renal Stem Cells

Functional recovery in acute renal failure is well known, and the adult kidney is generally recognized to have the capacity to regenerate and repair. The adult stem cells exist in the kidney, including slow-cycling cells, side population cells, CD133+ cells and rKS56 cells. However, in vivo differentiation of bone marrow-derived cells into renal tubular cells may not occur at all, or is at most a minor component of the repair process. Moreover, it is generally accepted that stem cells and multipotent cells contribute to the regenerative process by producing protective and regenerative factors rather than by directly differentiating to replace damaged cells. Therefore, for clinical regenerative medicine in kidney disease, the focus of stem cell biology will shift from multiple differentiation of cells or cell-therapy to multiple functions of the cells, such as the production of bone morphologic protein-7 and other regenerative factors (Hishikawa and Fujita, 2006).

Adult stem cells have been characterized in several tissues as a subpopulation of cells able to maintain generate, and replace terminally differentiated cells in response to physiological cell turnover or tissue injury. Little is known regarding the presence of stem cells in the adult kidney but it is documented that under certain conditions, such as the recovery from acute injury, the kidney can regenerate itself by increasing the proliferation of some resident cells. The origin of these cells is largely undefined; they are often considered to derive from resident renal stem or progenitor cells. Whether these immature cells are a subpopulation preserved from the early stage of nephrogenesis is still a matter of investigation and represents an attractive possibility. Moreover, the contribution of bone marrow-derived stem cells to renal cell turnover and regeneration has been suggested. In mice and humans, there is evidence that extrarenal cells of bone marrow origin take part in tubular epithelium regeneration. Injury to a target organ can be sensed by bone marrow stem cells that migrate to the site of damage, undergo differentiation, and promote

structural and functional repair. Hematopoietic stem cells are mobilized following ischemia/reperfusion and engrafted the kidney to differentiate into tubular epithelium in the areas of damage. The evidence that mesenchymal stem cells, by virtue of their renoprotective property, restore renal tubular structure and also ameliorate renal function during experimental acute renal failure provides opportunities for therapeutic intervention (Morigi, 2006).

Acute renal failure has 50-80% mortality and treatment options for this life-threatening disease are limited. Stem cells offer an exciting potential for kidney regeneration. This review discusses pathogenesis of acute renal failure resulting from ischemia-reperfusion injury and the role of stem cells in reversing or mitigating this disorder. Specifically, the issues of differentiation of kidney cells from embryonic stem cells and bone marrow stem cells, and whether adult kidney stem/progenitor cells exist in the postnatal kidney are discussed. Evidence to support the conclusion that intra-renal cells, including surviving tubular epithelial cells and potential renal stem/progenitor cells, are the main source for renal regeneration is provided. Future research in selecting the type(s) of stem cells and optimizing the dose, frequency and route of administration of the cells will be fundamental in successful cell replacement therapy in acute renal failure (Lin, 2006).

Repair of inflammatory and/or ischemic renal injury involves endothelial, mesangial and epithelial regeneration. These structures may be rebuilt by resident progenitor cells and bone marrow-derived stem cells. Resident progenitor cells in adult kidney have not yet been conclusively identified. They are likely to be slowly cycling cells located mainly in the outer medulla and renal papilla. In glomerulonephritis with mesangiolysis, mesangial regeneration involves progenitor cells migrating from the juxtaglomerular apparatus and also bone marrow-derived cells. In acute ischemic renal failure, epithelial regeneration of proximal tubules results from the migration, proliferation and differentiation of resident progenitor cells; bone marrow-derived cells may play an accessory role. Molecular mechanisms underlying these repair processes could be targets for new therapeutic approaches (Baud, 2005).

Ischemia causes kidney tubular cell damage and abnormal renal function. The kidney is capable of morphological restoration of tubules and recovery of function. Recently, it has been suggested that cells repopulating the ischemically injured tubule derive from bone marrow stem cells. In GFP chimeras, some interstitial cells but not tubular cells express GFP after ischemic injury. More than 99% of those GFP interstitial cells are leukocytes. In female mice with male bone marrow, occasional tubular cells (0.06%) appeared to be positive for the Y chromosome, but deconvolution microscopy revealed these to be artifactual. In beta-gal chimeras, some tubular cells also appear to express beta-gal as assessed by X-gal staining, but following suppression of endogenous (mammalian) beta-gal, no tubular cells could be found that stain with X-gal after ischemic injury. Whereas there is an absence of bone marrow-derived tubular cells, many tubular cells expressed proliferating cell nuclear antigen, which is reflective of a high proliferative rate of endogenous surviving tubular cells. Upon i.v. injection of bone marrow mesenchymal stromal cells, postischemic functional renal impairment was reduced, but there was no evidence of differentiation of these cells into tubular cells of the kidney. Bone marrow-derived cells do not make a significant contribution to the restoration of epithelial integrity after an ischemic insult. It is likely that intrinsic tubular cell proliferation accounts for functionally significant replenishment of the tubular epithelium after ischemia (Duffield, 2005).

Acute renal failure (ARF) is a common disease with high morbidity and mortality. Recovery from ARF is dependent on the replacement of necrotic tubular cells with functional tubular epithelium. Recent advancement in developmental biology led to the discovery of immature mesenchymal stem cells (MSCs) in bone marrow and several established organs and to the definition of their potential in the recovery from tissue injury (Herrera, 2004).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is an area of intense investigation. Many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. Extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but and vast majority of tubular cells derive from an intrarenal source. Interstitial cells may represent another extratubular stem cell niche. It is not clear whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex,

medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells (Humphreys, 2006).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is currently an area of intense investigation. Studies from our laboratory and others have supported a model whereby many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. A third hypothesis is that extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but our work and work of others indicates that the vast majority of tubular cells derive from an intrarenal source. Recent evidence suggests that interstitial cells may represent another extratubular stem cell niche. The fundamental unanswered questions in this field include whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells. In this review we focus on our current understanding of the potential role of renal and extrarenal stem cells in repair of the adult kidney and highlight some of the controversies in this field (Humphreys, 2006).

The capacity of the kidney to regenerate functional tubules following episodes of acute injury is an important determinant of patient morbidity and mortality in the hospital setting. After severe injury or repeated episodes of injury, kidney recovery can be significantly impaired or even fail completely. Although significant advances have been made in the clinical management of such cases, there is no specific therapy that can improve the rate or effectiveness of the repair process. Recent studies have indicated that adult stem cells, either in the kidney itself or derived from the bone marrow, could participate in this repair process and might therefore be utilized clinically to treat acute renal failure. This review will focus on our current understanding of these stem cells, the controversies surrounding their in vivo capacity to repopulate the renal tubule, and further investigations that will be required before stem cell therapy can be considered for use in the clinical setting (Cantley, 2005).

While it remains unknown whether there is a stem cell in the adult kidney, characterization of the cell populations involved in renal repair and misrepair is allowing a new understanding of the mechanisms that are responsible for renal homeostasis (Oliver, 2004).

Ischemia-reperfusion injury (I/R injury) is a common cause of acute renal failure. Recovery from I/R injury requires renal tubular regeneration. Hematopoietic stem cells (HSC) have been shown to be capable of differentiating into hepatocytes, cardiac myocytes, gastrointestinal epithelial cells, and vascular endothelial cells during tissue repair. The current study tested the hypothesis that murine HSC can contribute to the regeneration of renal tubular epithelial cells after I/R injury (Lin, 2003).

The kidney has the ability to restore the structural and functional integrity of the proximal tubule, which undergoes extensive epithelial cell death after prolonged exposure to ischemia. Small numbers of peritubular endothelial cells to be derived from bone marrow cells that may serve in the repair process (Duffield, 2005).

Renal progenitor tubular cells [label-retaining cells (LRC)] are identified in normal kidneys by in vivo bromodeoxyuridine (BrdU) labeling. In normal and contralateral kidneys, LRC are observed scattering among tubular epithelial cells. After unilateral ureteral obstruction (UUO), the number of the LRC significantly increase, and most of them are positive for proliferating cell nuclear antigen (PCNA). In contrast, PCNA+ cells lacking BrdU label are rarely observed. LRC are not only in tubules but also in the interstitium after UUO. Laminin staining showed that a number of the LRC are adjacent to the destroyed tubular basement membrane. Some tubules, including LRC, lose the expression of E-cadherin after UUO. A large number of cell populations expressed vimentin, heat shock protein 47, or alpha-smooth muscle actin in the UUO kidneys, and each population contained LRC. None of the LRC is positive for these fibroblastic markers in contralateral kidneys. When renal tubules from BrdU-treated rats are cultured in the gel, some cells protruded from the periphery of the tubules and migrated into the gel. Most of these cells are BrdU+. Neither the total content of BrdU in the kidneys nor the number of LRC in bone marrow significantly is changed after UUO. LRC is a cell population that proliferates, migrates, and transdifferentiates into fibroblast-like cells during renal fibrosis (Yamashita, 2005).

8. Human Embryonic Stem Cell (hESC)

Scientific progress in human embryonic stem cell (hESC) research and increased funding make it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Several issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. To minimize the risk of hESC transplantation, donors of materials used to derive hESC lines will need to be recontacted to update their medical history and screening. Because of privacy concerns, such recontact needs to be discussed and agreed to at the time of donation, before new hESC lines are derived. Informed consent for Phase I clinical trials of hESC transplantation also raises ethical concerns. In previous Phase I trials of highly innovative interventions, allegations that trial participants had not really understood the risk and benefits caused delays in subsequent trials. Thus researchers should consider what information needs to be discussed during the consent process for hESC clinical trials and how to verify that participants have a realistic understanding of the study. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies.

Increased funding and continued scientific progress have opened a new era in the ethics of human embryonic stem cell (hESC) research. These developments will reframe the ethical debate, which to date has focused on the moral status of the embryo and the acceptability of using embryos for research purposes. Although such philosophical questions have not been resolved, the issue is no longer *if* hESC research should proceed, but rather *how* it should proceed. The rapid pace of research makes it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Some of these issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. Crucial issues concerning safety of hESC transplantation and the need to recontact donors of materials used to derive new hESC lines are best resolved when these materials are donated. In addition, informed consent for hESC transplantation Phase I clinical trials will present particular challenges, which will require modification of the usual consent process for clinical trials. Failure to address these ethical issues may delay or preclude clinical trials that will test whether interventions based on hESC are safe and effective.

8.1 The current Scientific, Ethical, and Policy Context of hESC Research

New hESC lines are needed if hESCs or their products are to be used for transplantation into humans. The twenty or so hESC lines approved for federally funded studies in 2001 by President Bush were derived using nonhuman feeder cells and serum and express the nonhuman antigen Neu5Gc. Thus, they would probably be immunologically rejected by the recipients unless this problem was remedied. Derivation of new hESC lines will be stimulated by the $3 billion in funding for stem cell research authorized by California voters in 2004. This measure will give priority to funding research that cannot be funded by NIH, which is currently the case for derivation of new hESC lines. Other states and private funders have followed suit in providing nonfederal support for hESC research. Outside of the U.S., hESC research is advancing vigorously. In May 2005, researchers from South Korea reported the derivation of 11 hESC lines using somatic cell nuclear transfer, demonstrating that technical obstacles to developing such stem cell lines can be overcome more readily than expected. In turn, such findings will stimulate further research.

Current ethical and policy guidelines for hESC research focus on the derivation of new hESC lines. In May 2005, a National Academy of Sciences (NAS) panel called for voluntary adoption of ethical guidelines in hESC research. Their recommendations included institutional oversight of hESC research protocols through Embryonic Stem Cell Research Oversight Committees (ESCROs), informed consent from donors of materials for new hESC lines, restrictions on payment to gamete donors, and guidelines for banking stem cells and documentation. The twenty-three NRC recommendations have been endorsed by academic and scientific organizations and adopted as interim regulations for research funded by the state of California. That same month, the FDA issued regulations on screening and testing donors of human cells, tissues, and cellular and tissue-based products (HCT/P). While valuable, these initial efforts do not address crucial ethical issues in clinical trials of hESC transplantation, which have important upstream implications for how hESC lines should be derived, as well as for the conduct of the trials themselves. Our analysis begins with the need both to protect participants in Phase I trials of hESC transplantation and to respect the confidentiality of donors of materials used for derivation of hESC lines. These ethical responsibilities need to be addressed during the initial process of donating materials for new hESC lines. Next we consider challenges confronting informed consent for Phase I trials of hESC transplantation. We present specific recommendations for resolving these ethical issues.

8.2 Balancing the Need to Protect Participants in Phase I Clinical Trials Against the Need to Respect Donors

The goal of Phase I clinical trials is to assess the safety and feasibility of the investigational intervention and to determine dosages for subsequent clinical trials. Direct therapeutic benefit, although hoped for, is unlikely in early trials, particularly if the first participants receive low doses. The guiding ethical principle of Phase I studies should be "Do no harm." This ethical responsibility to protect the subjects in Phase I trials has important implications for the derivation of hESC lines. A major safety concern is transmission of infectious agents or serious genetic conditions through transplanted hESC cells or products. The public will expect strong protections against diseases transmitted through hESC transplantation, just as it demands that blood transfusions and solid organ transplants be tested for very rare but serious communicable diseases. The May 2005 FDA regulations addressed possible transmission of communicable diseases by cell-based therapies, setting standards for screening and testing at the time of donation and for tracking transplanted materials back to the original donors. HCT/P must be linked through an identification code to the donor and to pertinent donor medical records. Although these requirements are necessary to protect recipients, we contend that they are not sufficient to adequately protect them.

A broader perspective on protecting recipients of transplanted hESC materials is needed because of several clinical features of hESC transplantation. First, there is likely to be a considerable time period between donation of biological materials used to derive hESC lines and clinical trials involving transplantation of hESCs or products from them. During this period, new risks may become apparent in the donors whose gametes were used to derive the hESC lines. Emerging infectious diseases with long latency periods, such as Creutzfeldt-Jakob Disease (CJD), may be identified, for which testing and screening were not available at time of donation. Polymorphisms and biomarkers associated with risk for specific diseases are being defined at a rapid pace. Second, in hESC transplantation, serious genetic conditions might also be transmitted, some of which may not have been apparent at the time the materials were donated. For instance, after donating, donors may develop cancer or a strong family history of cancer. Third, immunosuppressive drugs, which may be essential after cell transplantation to reduce rejection, will increase the risk of communicable diseases and cancer in recipients. Fourth, if hESC transplantation proves clinically effective, many patients may receive transplantation from a single hESC line over time. Hence many recipients may be at risk for diseases transmitted from donors. In order to safeguard recipients of hESC transplantation, researchers need to recontact persons whose gametes were used to derive the hESC lines at the time of clinical hESC transplantation trials to update information and perhaps do additional testing. Furthermore, if hESC transplantation becomes a proven clinical treatment, periodic updating of the clinical status of donors would be prudent.

How can screening and testing of donors of materials for hESC lines be updated in an ethically acceptable manner? The responsibility to protect hESC transplant recipients from harm must be balanced against a responsibility to respect donors and protect their confidentiality. To resolve these countervailing mandates, researchers will need to obtain permission to recontact donors if hESC cells or materials derived from their gametes or embryos will be used for transplantation. Researchers need to tell donors about the kinds of information or testing that might be requested later and the reasons the information is needed. Such permission for recontact needs to be obtained when materials are donated for research. Without this permission, it would be a serious invasion of privacy to later recontact the donors. Also, donors who had not agreed to be recontacted might object strongly to a subsequent contact, refuse to provide information about their interim medical history, or undergo additional testing. Previous reports on the consent process for donating gametes and embryos for hESC research have not discussed the issue of recontact in depth. Obtaining permission to recontact will undoubtedly complicate the consent process for donating embryos for hESC research. However, permission for recontact will likely minimize the disqualification of hESC lines late in the development process for use in transplantation studies because of inadequate follow-up with donors. Recontacting donors presents logistical challenges because donors may move and contact may be lost. It would be desirable to ask donors to provide contact information for a relative or friends who will know their new address should they move. Confidentiality must be carefully protected because breaches might subject donors to unwanted publicity or even harassment. Concerns that their identities will not be kept confidential may deter some individuals from agreeing to be recontacted. Because of the intense public interest in and contentiousness over hESC research, it would be prudent for researchers and research institutions to develop stringent mechanisms, extending beyond those employed in routine clinical care, in order to assure donors that their identity and contact information remain protected.

Recently, confidentiality of personal health care information has been violated through deliberate breaches by staff, through break-ins by computer hackers, and through loss or theft of laptop computers. Files containing the identities of persons whose gametes were used to derive hESC lines should be protected against such breaches through additional security measures. Any computer storing such files should be locked down in a secure room and password protected, with access limited to a minimum number of individuals on a strict "need-to-know" basis. Entry to the computer storage room should also be restricted by means of a card-key, or equivalent system, that records each entry. Audit trails of access to the information should be routinely monitored for inappropriate access. The files with identifiers should be copy-protected and double encrypted, with one of the keys held by a high-ranking institutional official who is not involved in stem cell research. The computer storing these data should not be connected to the Internet. To protect information from subpoena, investigators should obtain a federal Certificate of Confidentiality. Human factors in breaches of confidentiality should also be considered. Personnel who have access to these identifiers might receive additional background checks, interviews, and training. The personnel responsible for maintaining this confidential database and contacting any donor should not be part of a hESC research team. Funders of hESC research and IRBs or ESCROs that oversee hESC research should ensure that appropriate provisions for recontact and confidentiality are in place. The IRB should review and approve any requests for recontact of donors. The ethical reasons for these provisions are sufficiently compelling that materials donated without explicit permission for recontact should not be used to develop hESC lines for transplantation, lest the safety of recipients or privacy of the donors be compromised.

8.3 Informed consent for recipients of hESC transplantation in Phase I clinical trials

Current procedures for obtaining informed consent are likely to be inadequate to address particular issues faced by recipients of hESC transplantation in Phase I clinical trials. Because the matter is complex and any changes in policy will need careful consideration, discussions of the consent process need to begin now. Problems with informed consent commonly occur in clinical trials. Participants in cancer clinical trials commonly expect that they will benefit personally from the trial, even though the primary purpose of Phase I trials is to test safety rather than efficacy. This tendency to view clinical research as providing a personal benefit has been termed the "therapeutic misconception". Analyses of consent forms suggest that such misunderstandings in cancer clinical trials do not reflect information in the consent forms. Indeed, cancer patients seeking therapeutic benefit may decide to enroll in a clinical trial before they meet the research staff, before they learn about the risks and benefits of the study or read a consent form.

One study of the consent process in gene transfer clinical trials found that researchers' descriptions of the direct benefit to participants in Phase I trials commonly were vague, ambiguous, and indeterminate. Some investigators try to balance hope and practical reality, for example believing that "if we've done our job right, they don't expect it, but they hope for it". This study concluded that "there is no clear resolution to the underlying normative question: what should investigators communicate about the potential for direct benefit to subjects in early phase clinical research"? The authors suggested that "this dilemma cannot be addressed by individual PIs alone, but must be acknowledged and openly discussed by the scientific community". Investigators need to determine how to develop ways to present clearly to participants such issues as promising preclinical evidence, the lack of power to detect benefit in Phase I studies, and the clinical significance of surrogate endpoints. In Phase I trials of hESC transplantation, guidelines for describing the likely direct benefits to participants similarly would require wide discussion, not only by scientists but also by public representatives. Participants receiving hESC transplantation in Phase I trials might overestimate the benefits and underestimate the risks for several reasons. The therapeutic misconception may be particularly prominent because the scientific rationale for hESC transplantation and preclinical results may seem compelling. In addition, press accounts of stem cell research, which typically have emphasized its potential to treat currently incurable diseases, may reinforce unrealistic hopes. Participants in Phase I trials may not appreciate that there is a possibility that hESC transplantation might make their condition worse. In previous clinical trials of transplantation of fetal dopamine neurons into persons with Parkinson's, transplanted cells failed to improve clinical outcomes. Indeed, late disabling dyskinesias developed in about 15% of patients receiving transplantation, with some patients needing ablative surgery to relieve these adverse events. Although the transplanted cells localized to the target areas of the brain, engrafted, and functioned to produce the intended neurotransmitters, appropriately regulated physiologic function was not achieved.

Several measures may reduce the therapeutic misconception in recipients of hESC transplantation in Phase I clinical trials. First, researchers should frame their discussions with participants in the context of publicity about the potential for hESC to treat serious diseases. Researchers need to communicate the distinction between the long-term hope for such effective treatments and the uncertainty inherent in any Phase I trial. Participants in Phase I studies need to understand that hESCs have never been tried before in humans for the specific study purposes, that researchers do not know whether they will work as hoped, and that in fact the great majority of participants in Phase I studies do not receive any direct benefit. Second, investigators in hESC clinical trials must discuss a broader range of information with potential participants than in other clinical trials. Informed consent requires researchers to discuss with potential participants information that is pertinent to their decision to volunteer for the clinical trial. Generally, the relevant information concerns the nature of the intervention being studied and the medical risks and prospective benefits. However, in hESC transplantation, non-medical issues may be prominent or even decisive for some participants. Individuals who regard the embryo as having the moral status of a person would likely have strong objections to receiving hESC transplants. Even though this intervention might benefit them medically, these individuals might regard it as collaborating with or taking advantage of an immoral action, and thus tacitly supporting it. Researchers need to appreciate that views of hESC research are not monolithic and may change over time. Indeed, some individuals who are strong advocates of pro-life positions and opponents of abortion regard the blastocyst as a potential person, not an actual person. In this latter view, hESC transplantation is morally acceptable. Researchers in clinical trials of hESC transplantation should inform eligible participants that transplanted materials originated from human embryos and help them to think through the ethical implications and clarify their personal beliefs about this research. The therapeutic misconception and beliefs about the moral acceptability of hESC research may interact in complex ways. It is possible that people who mistakenly believe that hESC Phase I clinical trials will benefit them medically may, in their eagerness to obtain treatment for a serious medical condition, overlook the origin of transplanted material. If they fail to gain clinical benefit from the clinical trial, they may then have second thoughts about their decision to accept such an intervention. Third, and most importantly, researchers should verify that participants have a realistic understanding of the study. The crucial ethical issue about informed consent is not what researchers disclose in consent forms or discussions, but rather what the participants in clinical trials understand. In other contexts, some researchers have ensured that participants understand the key features of the trial by testing their comprehension. In controversial HIV clinical trials in developing countries, where it has been alleged that participants did not understand the trial, some researchers are now testing each participant in such trials to be sure he or she understands the essential features of the research as part of the consent process. Direct assessment of participants' understanding of the study, in contexts where misunderstandings are likely, has also been recommended by several national panels. We urge that such tests of comprehension be routine in clinical trials of hESC transplantation. Controversies about the consent process might lead to delays in clinical trials of cutting-edge interventions. In early clinical trials of organ transplantation, the implantable totally artificial heart, and gene transfer, the occurrence of serious adverse events led to allegations that study participants had not truly understood the nature of the research. In turn, these concerns about consent contributed to delays in subsequent trials. Assessing the comprehension of participants would reduce or preclude post-hoc criticisms that hESC recipients did not understand the essential features of the Phase I trial. To strengthen the informed consent process in trials of hESC transplantation, stakeholders should develop consensus best practice recommendations for informing potential participants about early hESC clinical trials and for assessing participants' comprehension of key features of these trials. These stakeholders include researchers, public representatives, advocacy groups, government officials, and members of institutional hESC oversight committees. Because such consensus guidelines need to be in place by the time such clinical trials are proposed, these stakeholder meetings should be convened now. hESC clinical trials raise other important ethical questions. What kinds of in vit2o studies must be done to characterize hESC and document karyotype, epigenetic status, cell cycle parameters, and differentiation potential? What kinds of preclinical and animal studies should be required before hESC transplantation is attempted in humans? What long-term follow-up of participants should be carried out, and how can data on adverse events be pooled across different protocols? Who will pay for such long-term follow-up, since many Phase I trials will not lead to commercial products? To the extent that disagreements over these questions might delay clinical trials, these issues also need to be addressed in advance. In conclusion, for hESC to fulfill its promise as therapy, a chain of activities needs to be established, including funding, basic science, and clinical trials. Recent events have increased funding and shown that the science may proceed

rapidly. But a chain is only as strong as its weakest link. Attention to ethical issues raised by clinical trials is an essential part of the chain. The issues we have discussed are based on lessons from previous experience with related but not identical fields; invariably other unforeseeable issues will arise. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies (Bernard, 2005).

9. Selected Protocols for Stem Cell Researches

9.1 INFT2 Protocol

Hematologic malignancies (blood cell cancers) in very young children are hard to treat with standard doses of chemotherapy (anti-cancer drugs). Stem cell transplantation (infusion of healthy blood forming cells) has been used but has not always been successful. The best donor of stem cells is a sibling (brother or sister) who is a match (the sibling's cells match the subject's immune type, or HLA type). But few very young children with leukemia have a matched sibling donor. This research study is for those children who do not have a matched sibling donor. In this study, a parent will be the stem cell donor. Using a parent donor (a parent donor is a partial match for the subject's HLA type) increases the risk of graft-versus-host disease (GVHD). GVHD occurs when the donor cells (the graft) recognize that the body tissues of the child (the host) are different. Because severe GVHD can be life-threatening, the parent's stem cells will be filtered using a machine called the CliniMACS system, which removes the cells that cause GVHD. This system has not been approved by the Food and Drug Administration (FDA) and is considered experimental. In addition to the stem cell transplant, parent donor natural killer (NK) cells will be given. NK cells are special cells in the immune system (the body organs and cells that defend the body against infection and disease) that target cancer cells. NK cells may help donor cells to grow and may reduce the chance of GVHD. In this experimental treatment, chemotherapy will be used in addition to the stem cell and NK cell transplants. It is unknown if these treatments will work better than the treatments now being used to treat very young children with hematologic malignancies. **(**Leung, 2007**)**

9.2 OPBMT2 Protocol

Malignant osteopetrosis **is a** genetic disease in which cells in the bone tissue (osteoclasts) do not function properly. These cells are unable to perform their biological job of breaking down old bone tissue as new bone tissue is being made. This causes the bone tissue to build up, producing thick bones that do not work properly and causing the child to lose his/her bone marrow space, where red cells, platelets, and white cells are made.

Stem cell transplantation from an allogeneic donor is the only known cure for this disease. Stem cells are immature cells found in the bone marrow that can grow into other kinds of cells. An allogeneic donor is another person who provides the stem cells.

There are three types of donors:

(1) A matched sibling donor (brother or sister) is the ideal treatment, but is not possible for the majority of patients.

(2) A matched unrelated donor may also be used, but finding such a match may take several months. During this time the disease may get worse; the child may need red cell or platelet transfusions as the child may be unable to make these cells and permanent damage to vision and hearing may occur.

(3) A haploidentical parental donor (a mother or father), has not been studied previously as a treatment for malignant osteopetrosis.

This study is designed to use a haploidentical parental donor in the event that a matched sibling donor is unavailable. Using a parental donor would enable transplantation earlier in the disease process than waiting for a matched unrelated donor. This might reduce the chance of the disease getting worse before the transplant is done. With a parental donor, the risk of graft rejection (the patient's body will not accept and allow donated cells to grow) may be greater than the risk of rejection using a matched sibling donor.

The purpose of this study is to learn more about the cause and treatment of malignant osteopetrosis. It is designed to determine if children with malignant osteopetrosis can properly accept a parental donor

transplant and to study the genetic (characteristics carried by genes) factors which cause the disease (Kasow, 2007).

9.3 SCDHAP Protocol

Sickle cell disease is a lifelong blood condition that can cause damage to the brain and other organs of the body. Children may develop severe clinical states with recurrent vaso-occlusive crises (VOC) which can cause severe pain, acute chest syndrome (ACS) and/or stroke. Treatment may include blood transfusions which may be required to prevent some of the conditions caused by this disease. Unfortunately, blood transfusions can cause iron overload, which can lead to severe and sometimes fatal complications.

Stem cells are young blood cells that can grow to make new blood cells such as white blood cells that help fight infections, platelets that help the blood to clot, and red blood cells that carry oxygen to the vital organs of the body. These cells may be taken from one individual (donor) and given to another (recipient). These stem cells, when placed in the body of the recipient, travel through the body to the bone marrow space and begin to grow and make new blood cells.

A stem cell transplant has been shown to help, and possibly cure, patients with sickle cell disease. Stem cells taken from a brother or sister may provide bone marrow that is a perfect match (same tissue type) for the recipient. Unfortunately, only about 10-20% of sickle cell patients have a matched sibling donor. Stem cells from partially matched (partial tissue match) family members have been tried with a few children with sickle cell disease. The risk and benefits of these types of transplants are not as well known as transplants using a matched donor. When children with sickle cell disease have no matched brother or sister donor, allogeneic transplants are a possible treatment available for these patients (Paul Woodard, 2007)

9.4 SCT521 (COG # ASCT0521) Protocol

Idiopathic pneumonia syndrome is a complication that may occur in children who have had a stem cell transplant. Often patients with pneumonia have a cough and chest pain, are short of breath, or require oxygen to help them breathe. In some transplant patients, pneumonia is caused by a bacteria or virus. However, with idiopathic pneumonia syndrome, pneumonia occurs in the absence of infection. Despite corticosteroids and supportive care, this condition may be fatal.

This research study will use a drug named etanercept. The drug has been approved by the Food and Drug Administration (FDA) for the treatment of certain joint or skin conditions in children over 4 years of age. Etanercept works by blocking the effects of a protein known as Tumor Necrosis Factor (TNF). TNF has been found in lung fluid from patients with idiopathic pneumonia syndrome. TNF may be involved in the development of lung injury in idiopathic pneumonia syndrome. An earlier study has determined`the largest amount of etanercept that can be given without causing bad effects. A small research study has been done with adults and children with idiopathic pneumonia syndrome. Etanercept was found to be safe, and several patients had improvement in their breathing (Madden, 2007).

10. Brief Descriptions of Stem Cell Facts

Mouse embryonic stem cells were first discovered in 1981. Since then, they have been an invaluable tool of modern biology and medical research. They have provided models to study diseases, they have brought about the discovery of many genes associated with diseases and they have been used to cure certain human disorders in animal models. After 20 years of exciting research, the mouse embryonic stem cell has helped to establish the value of these cells in *regenerative medicine*, which is the creation of cells or organs to replace tissues lost to disease or injury. The discovery of *human* embryonic stem cells in 1998 triggered important ethical controversy and debate, yet scientists are convinced that they hold enormous potential for clinical applications. Many diseases plaguing the modern world may be improved, or even cured, with therapies using human stem cells. Whether human embryonic stem cells or adult stem cells are used in future therapies will depend on the type of disease or injury. There are specific advantages for each stem cell type. Thanks to the ease of growing them in the laboratory, human embryonic stem cells may one day become the source of artificial organs. Or scientists might one day be able to mobilize one's own adult stem cells to repair tissue damage caused by trauma, disease, and even aging. To reach such goals, both human embryonic and adult stem cells will have to be extensively studied. The complementary information acquired from studying both stem cell types is the key to unlocking their full potential.

10.1 What Are Stem Cells?

A stem cell is the base building block of an entire family of cells that make up any organ. A common trait of stem cells is that they can maintain themselves indefinitely in a stem cell state, which is referred to as "self-renewal," while also producing — through division — more specialized cells. For example, the blood stem cell can produce all the cells in the blood, including the red blood cells, white blood cells and platelets.

10.2 Who Needs Stem Cells?

Harnessing the power of human stem cells will revolutionize our health, our lives, and our society. In principle, any affliction involving the loss of cells, including many diseases, injuries and even aging, could be treated with stem cells. In the United States alone, more than 100 million people could benefit from therapies derived from stem cell research.

10.3 Adult Stem Cells

Adult stem cells are more specialized stem cells living in the majority of tissues and organs in our bodies and generate the mature cell types within that tissue or organ. In tissues where adult stem cells have been found, they are extremely rare and very difficult to isolate. Once isolated, adult stem cells grow poorly in culture, and it is difficult to obtain enough of these cells for use in clinical trials. In addition, access to the tissues harboring these cells is problematic since most human tissue is not easily available. Two readily available sources of human adult stem cells are the bone marrow and the umbilical cord blood. In both these tissues are blood stem cells, as well as other rare types of stem cells, which can produce bone, muscle, blood vessels, heart cells and possibly more.

10.4 Adult Stem Cells in the Clinic

The majority of stem cell clinical trials now underway use blood stem cells from the bone marrow or umbilical cord blood to treat blood disorders or diseases, such as leukemia, different types of anemia, systemic lupus, and certain other autoimmune diseases or deficiencies. A handful of clinical trials are evaluating the use of one's own bone marrow stem cells to repair heart tissue and to improve blood flow or to help to repair bone and cartilage. Other adult stem cells being explored for use in the clinic include stem cells in the eye and the skin. Adult stem cells are also thought to play a role in tissue transplants that have been performed for several years. For example, insulin-producing cells for type I diabetes, fetal neurons for Parkinson's disease, and skin for bladder reconstruction have been transplanted successfully. It is possible that in cases where long-term regeneration has been achieved, stem cells contained in these tissues have contributed to regeneration. The widespread use of adult stem cell-derived therapies and treatments is complicated by several factors. First, available human tissue is scarce, with only 6000 donors/year for more than 100 million Americans that could benefit from cellular therapy. Second, immune rejection caused by not using one's own cells or tissue is a problem. On the other hand, using one's own cells or tissue may become a problem for older patients, as evidence has been accumulating that adult stem cells age during the life of the body and lose their potential. Thus, stem cells isolated from a young adult may have a greater potential to produce numerous daughter cells than the cells of an older person.

10.5 Embryonic Stem Cells

Human embryonic stem cells are like a blank slate and can produce all the cells of the body. They are obtained from the ICM (inner cell mass) of the blastocyst. The blastocyst is a very early stage of human development, which forms about 5 days after fertilization of an egg. It is approximately 1/10 the size of the head of a pin, almost invisible to the eye, and it has not yet implanted into the uterus.

Once the blastocyst has implanted and a normal pregnancy can be detected, it is too late to derive human embryonic stem cells from the embryo. At the blastocyst stage, organ formation has not started and more specialized cells are not yet present, not even the beginning of the nervous system. To obtain human embryonic stem cells, blastocysts created in culture for *in vitro fertilization* (IVF) treatment by combining sperm and egg in a dish, are used. If they are not implanted into the uterus, the blastocysts are either discarded or frozen for later fertility cycles. They can also be donated to other patients or to research. If not donated, they will stay in the freezer as long as the storage fees are paid, otherwise they will be discarded. Because the cells obtained from the blastocyst have not yet specialized, they are considered highly valuable. They can generate cells that go on to form all the body's tissues and organs.

10.6 Why Are Embryonic Stem Cells So Valuable?

While grown in a dish, human embryonic stem cells can maintain their *"stem-cellness"* and provide an unlimited supply of more stem cells, as well as specialized cells that can be used for experiments and for the development of therapies. Apart from their potential to treat or cure diseases, human embryonic stem cells also provide a model to study very early human development and some of the disorders that lead to birth defects and childhood cancers. Many of these disorders develop in early pregnancy and are impossible to study in humans. Also, human embryonic stem cells also can be used to examine the genes that are turned "on" or "off" as stem cells generate more specialized cell types, permitting a unique understanding of the genetics of human development. The specialized cells derived from human embryonic stem cells also can be used to study the effectiveness of potential new drugs to treat diseases. This provides a human cellular model and can reduce animal experimentation and drug development costs. Additionally, embryonic stem cells can be derived from human blastocysts with specific genetic abnormalities. These types of blastocysts are identified through genetic diagnosis during IVF treatment, to screen out genetically abnormal blastocysts, and are usually discarded. The stem cells from them can provide a unique resource to understand genetic diseases and to develop cures. Human embryonic stem cells also could be used to understand the origin or causes of various diseases such as Alzheimer's disease or Parkinson's disease, which are currently unknown. Stem cells derived through *nuclear transfer* (more info below) from patients with such afflictions would provide special tools to study these diseases and possibly develop drugs for treatments.

10.7 Embryonic Stem Cells in the Clinic

Embryonic stem cells have not yet been used in treating humans. But numerous animal studies have shown that many of the specialized cells derived from them can indeed integrate into damaged tissues and function properly. Thus, diseases such as myocardial infarction, severe immune deficiency, diabetes, Parkinson's disease, spinal cord injury, and demyelination have been successfully treated in animal models. But the pathway from animal models to the clinic is still complex and burdened with obstacles to be overcome. First, not all specialized cells derived from human embryonic stem cells have been shown to integrate into animal tissue and function properly. This can be due to the poor quality of the specialized cells derived in culture, or to a lack of adequate communication between the human cells and the animal environment in which they are placed. Then there is the problem of scaling up to yield enough of the specialized cells to treat a human, since this requires many more cells than to treat a tiny mouse. Such cells will have to be produced under specific conditions to ensure safety for use in patients. Most human embryonic stem cells are still grown on a layer of mouse feeder cells, a potential source of contamination. Last, there's the problem of immune rejection by the patient. While the drugs used in the organ transplantation field to suppress immune rejection have been improved over the years, rejection is still a major problem.

11. Techniques of Human Embryonic Stem Cells

11.1 Nuclear Transfer to Generate Stem Cells

Immune rejection of transplanted stem cells could be avoided if the therapeutic cells derived from the human embryonic stem cells express a patient's own genes and proteins. A method to generate these types of stem cells is by *nuclear transfer*. The nuclear transfer technique is similar to the process of generating a blastocyst from the fertilization of an egg by a sperm cell; however, in this process the DNA in an egg is exchanged for the DNA from a cell of the patient. The egg is then coaxed to divide in a culture dish into a blastocyst. The human embryonic stem cells derived from this blastocyst will be an identical genetic match to the patient and can provide "customized" replacement cells for any disorder.

11.2 Blastocyst Develops into a Living Clone

As long as the blastocyst is not implanted into a uterus, it cannot develop further into a living clone of the patient. If the blastocyst is implanted, it is possible that a live offspring could be born (so-called *reproductive cloning*). But based on animal models of reproductive cloning, the procedure is very inefficient; over 95%of the clones die before birth, and those that do survive have serious genetic and biological problems. Thus, medically it is irresponsible to consider reproductive cloning for humans. It is also morally and ethically unacceptable.

12. Eternal Life

The production of functional male gametes is dependent on the continuous activity of germline stem cells. The availability of a transplantation assay system to unequivocally identify male germline stem cells has allowed their in vitro culture, cryopreservation, and genetic modification. Moreover, the system has enabled the identification of conditions and factors involved in stem cell self-renewal, the foundation of spermatogenesis, and the production of spermatozoa. The increased knowledge about these cells is also of great potential practical value, for example, for the possible cryopreservation of stem cells from boys undergoing treatment for cancer to safeguard their germ line

According to Greek mythology, the hapless mortal Tithonus mistakenly asked the goddess Eos to confer eternal life rather than eternal youth, and he thus found himself condemned to immortal decrepitude. A new report suggests that if Tithonus had cut a side deal with Dionysus, the god of wine, he might have fared much better.

The study knits together threads of recent molecular research on aging, the venerable antiaging strategy of calorie restriction, and, surprisingly, the health benefits of moderate tippling. David Sinclair of Harvard Medical School in Boston and colleagues identify several naturally occurring small molecules that extend the life of yeast cells by approximately 70% and offer some protection to cultured human cells exposed to radiation. The molecules activate genes known to extend life span in laboratory animals. They belong to a family of chemicals known as polyphenols, some of which are prominent components of grapes, red wine, olive oil, and other foods.

The work by Sinclair and collaborators at the biotech firm BIOMOL Research Laboratories in Plymouth Meeting, Pennsylvania, including Konrad Howitz, is the latest in an increasingly hot field exploring the molecular biology of calorie restriction, a phenomenon first demonstrated in the 1930s. Laboratory rats fed a limited diet live about 40% longer than normal and are resistant to many chronic illnesses typical of aging. The observations have been replicated in yeast, fruit flies, nematodes, fish, spiders, and mice, with hints from ongoing experiments that they hold true for primates. These findings have fueled interest in understanding how calorie restriction works--and an increasingly spirited search for molecules that might mimic the process without requiring a draconian diet.

Research in the Massachusetts Institute of Technology laboratory of Leonard Guarente, for example, has shown that increasing the activity of a single gene, called *SIR2*, can extend the life span of yeast. And without the gene, calorie restriction doesn't prolong life. The new research shows that certain molecules activate *SIR2* in yeast, as well as an analogous gene, *SIRT1*, in human cells. Sinclair says that preliminary data from experiments in nematodes and fruit flies are "encouraging," in terms of whether similar activation of *SIR*-like genes, known collectively as sirtuins, can occur in those organisms, too. The study "establishes that you can get activation of *SIR2*," says Guarente, who has co-founded a company called Elixir Pharmaceuticals, which is searching for drugs that target the Sir pathway.

Working with colleagues at Harvard, BIOMOL researchers began screening a library of compounds about 2 years ago for molecules that trigger *SIRT1* activity. The initial screen yielded two polyphenols, quercetin (found in apples and tea) and piceatannol. The team then searched for other molecules with similar structures. That canvass yielded another 15 compounds, the most potent of which turned out to be resveratrol, found in grapes and red wine. It increased *SIRT1* activity 13-fold, the team reports online 24 August in *Nature*.

Resveratrol's *SIRT1*-activating power adds another dimension to the work, because it suggests a link to the so-called French paradox, the observation that despite a high-fat diet, people in France suffer about 40% less cardiovascular disease than expected; epidemiologists have linked this effect to the moderate consumption of red wine. Sinclair and colleagues speculate that these benefits may derive from activation of *SIR*-like genes. Increased *SIRT1* activity in human cells seems to blunt the activity of the tumor-suppressor gene *p53*, blocking programmed cell death. Sinclair suggests that the *SIR*-activating compounds buy time for cells to heal themselves rather than commit suicide.

In addition to its immediate implications for aging and life extension, the new work bolsters the notion that there is an evolutionarily conserved mechanism to stall the aging process during times of stress, such as when food is scarce. It also raises the possibility that the sirtuin-activating compounds reflect an interaction between plant and animal species. According to this hypothesis, which Sinclair calls "xenohormesis," plants increase their own production of polyphenols in response to environmental stresses such as drought, and that message of impending crisis may be passed on to animals that eat the plants. "Other unrelated, nonplant species can get chemical clues from the plant world," Sinclair says, "which causes them to mount their own defense response." Alternatively, he adds, the plant compounds may simply be similar to analogous, unidentified molecules in human biology.

Richard Weindruch of the University of Wisconsin, Madison, who is conducting calorie-restriction experiments in monkeys and other animals, applauds the new report but adds, "I think one needs to be very cautious about making dramatic leaps from the yeast model into mammals." He notes that it was unclear, for example, whether resveratrol affected the aging process in the kind of cells in the heart and brain that are particularly susceptible to degeneration with age.

"It's kind of romantic that red wine contains something that could extend your longevity, don't you think?" says Cynthia Kenyon, who researches aging at the University of California, San Francisco, after seeing the data presented at a meeting in Switzerland last week. But the results have not caused Sinclair to renegotiate his relationship with Dionysus. "I'd already increased my red wine consumption prior to this discovery," he confesses with a laugh (Hall, 2003).

13. Debates on Stem Cell Research

There are a lot of debates on the stem cell research. There most important concerns for the stem cell research and application are:

(1) As the stem cell has the totipotent property to form any kind of cells, it is very easy for the stem cell to form can cancer cell. Also, it has the potential danger possibility to create some kind of disease that there no way to cure in a current human technological condition. If it loses control, it is danger for the whole human society.

(2) The stem cell can be a potential weapon if it is used by terrorists.

(3) From the religious aspects, it is a critical topic to use stem cell at research, especially to use embryonic stem cell, as the embryo can be considered as the individual living body from some religious spirit. At this point, some religious followers persist that to take cells, tissues and organs from an embryo is same as to take that cells, tissues and organs from an individual alive living body, and destroy the embryo is same as to kill the living individual. It is a big ethnical question in the religious stage.

(4) The stem cell research and application is a critical topic in the political stage, as the politician must consider the public opinions, no matter the politician agree the opinions or not, and no matter the opinions are right or wrong.

(5) Up to now, the technique ability is not enough for the scientists to full control the stem cell research and application, and too many thing we do not know the field.

Stem cell research is a high-tech question and the people involved in this rebates should have certain scientific knowledge on the stem cell. However, it is OK for the politicians or religionists to show their opinions on any topic they are interested in, but not suitable for them to make decisions (or make laws) that will significantly influence the scientific research as this field the politicians or religionists are not specialized. Such as, it is not suitable for the American President George W. Bush to show the power in the stem cell research. It is scientists' job. When politics and science collide, science should do scientific way, rather political way. Major ethical and scientific debates surround the potential of stem cells to radically alter therapies in health care (Williams, 2005).

No matter how much debate the stem cell research and application are facing, nobody can stop these researches and applications. One country or community can stop these researches and applications within its boundary through its political or its religious power, but it has very weak power to control the researches and application outside it boundary. No matter it is United States with its super military power, or United Union through its super international influence ability, or Pope through its super religious power, nobody can stop stem cell research and application right now. There are too many danger things in stem cell exploring, and there are too many benefits from the stem cell facts. The attractions are too strong that are luring people to much.

14. Stem Cell Glossary

(1) **Adult stem cells:** Stem cells found in different tissues of the developed, adult organism that remain in an undifferentiated, or unspecialized, state. These stem cells can give rise to specialized cell types of the tissue from which they came, i.e., a heart stem cell can give rise to a functional heart muscle cell, but it is still unclear whether they can give rise to all different cell types of the body.

(2) **Blastocyst:** A very early embryo consisting of approximately 150 cells. It contains the inner cell mass, from which embryonic stem cells are derived, and an outer layer of cells called the trophoblast that forms the placenta.

(3) **Cell line:** Cells that can be maintained and grown in culture and display an immortal or indefinite life span.
(4) **Differentiation:** The process of development with an increase in the level of organization or complexity of a cell or tissue, accompanied with a more specialized function.
(5) **Embryo:** The product of a fertilized egg, from the zygote until the fetal stage.
(6) **Embryonic stem cell:** Also called ES cells, embryonic stem cells are cells derived from the inner cell mass of developing blastocysts. An ES cell is self-renewing (can replicate itself), pluripotent (can form all cell types found in the body) and theoretically is immortal.
(7) **In vitro fertilization:** A procedure where an egg cell and sperm cells are brought together in a dish so that a sperm cell can fertilize the egg. The resulting fertilized egg, called a zygote, will start dividing and after a several divisions, forms the embryo that can be implanted into the womb of a woman and give rise to pregnancy.
(8) **Mesenchymal stem cell:** Also known as bone marrow stromal cells, mesenchymal stem cells are rare cells, mainly found in the bone marrow, which can give rise to a large number of tissue types such as bone, cartilage, fat tissue, and connective tissue.
(9) **Multipotent stem cells:** Stem cells whose progeny are of multiple differentiated cell types, but all within a particular tissue, organ, or physiological system. For example, blood-forming (hematopoietic) stem cells are single multipotent cells that can produce all cell types that are normal components of the blood.
(10) **Nucleus:** A part of the cell, situated more or less in the middle of the cell, which is surrounded by a specialized membrane and contains the DNA of the cell, which is the genetic, inherited material of cells.
(11) **Plasticity:** A phenomenon used to describe a cell that is capable of becoming a specialized cell type of different tissue.
(12) **Pluripotent stem cells:** Stem cells that can become all the cell types that are found in an implanted embryo, fetus, or developed organism.
(13) **Progenitor cell:** An early descendant of a stem cell that can differentiate, but cannot renew itself. By contrast, a stem cell can renew itself (make more stem cells by cell division) or differentiate (divide and with each cell division evolve more and more into different types of cells).
(14) **Regenerative medicine:** Medical interventions that aim to repair damaged organs, most often by using stem cells to replace cells and tissues damaged by aging and by disease.
(15) **Reproductive cloning:** Somatic cell nuclear transfer used for the production of a fetus and delivery of a live offspring that is genetically identical the donor of the somatic cell DNA.
(16) **Somatic cells:** All the cells within the developing or developed organism with the exception of germline (egg and sperm) cells.
(17) **Stem cells:** Cells that have both the capacity to self-renew (make more stem cells by cell division) and to differentiate into mature, specialized cells.
(18) **Therapeutic cloning:** Somatic cell nuclear transfer for the isolation of embryonic stem cells. The embryonic stem cells are derived from the blastocyst (before it becomes a fetus) and can be instructed to form particular cell types (e.g. heart muscle) to be implanted into damaged tissue (e.g. heart) to restore its function. If the stem cells are placed back into the individual who gave the DNA for the somatic cell nuclear transfer, the embryonic stem cells and their derivatives are genetically identical and thus immunocompatible (they will not be rejected).
(19) **Transdifferentiation:** The ability of a particular cell of one tissue, organ or system, including stem or progenitor cells, to differentiate into a cell type characteristic of another tissue, organ, or system; e.g., blood stem cells changing to liver cells.
(20) **Transplantation biology:** The science that studies the transplantation of organs and cells. Transplantation biologists investigate scientific questions to understand why foreign tissues and organs are rejected, the way transplanted organs function in the recipient, how this function can be maintained or improved, and how the organ to be transplanted should be handled to obtain optimal results.
(21) **Umbilical cord stem cells:** Hematopoietic stem cells are present in the blood of the umbilical cord during and shortly after delivery. These stem cells are in the blood at the time of delivery, because they move from the liver, where blood-formation takes place during fetal life, to the bone marrow, where blood is made after birth. Umbilical cord stem cells are similar to stem cells that reside in

bone marrow, and can be used for the treatment of leukemia, and other diseases of the blood. Efforts are now being undertaken to collect these cells and store them in freezers for later use.

(22) **Zygote:** The cell that results from the union of sperm and egg during fertilization. Cell division begins after the zygote forms.

Correspondence to:
Ma Hongbao, Ph.D.
Brookdale University Hospital Medical Center
Brooklyn, New York 11212, USA
Email: hongbao@gmail.com
Telephone: 347-789-4323

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